Concentration and Purification of Hepatitis B Antigen with Polyethylene Glycol and Polyelectrolyte 60, a Cross-Linked Copolymer of Isobutylene Maleic Anhydride

EDDA DE RIZZO, RAMAYAN PANDEY, CRAIG WALLIS, AND JOSEPH L. MELNICK
Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas 77025

Received for publication 26 April 1972

A simple two-step procedure is described for the concentration and purification of hepatitis B antigen. The antigen is precipitated by polyethylene glycol and then adsorbed on and eluted from an insoluble polyelectrolyte, polyelectrolyte 60, which is a cross-linked copolymer of isobutylene maleic anhydride.

Concentration and purification of hepatitis B antigen (HB Ag) has been accomplished by density gradient centrifugation and column chromatography, or both (3–8, 10). However, the published procedures are laborious and time consuming. Many viruses can be concentrated on polyelectrolyte 60 (PE 60), an insoluble copolymer belonging to the isobutylene maleic anhydride class (13). However, PE 60 will not adsorb virus particles in the presence of serum proteins, especially albumin and gamma globulins. Polson et al. (11) have shown that polyethylene glycol (PEG) will precipitate from plasma the alpha globulins, with which HB Ag migrates electrophoretically (1, 2). Thus, partial purification of HB Ag by PEG would be expected to yield a concentrate low in albumin and gamma globulins, which would favor HB Ag adsorption on PE 60. The current study was undertaken to develop a two-step method for concentration and purification of HB Ag that would take advantage of the properties of PEG and PE 60 and would not be dependent upon expensive apparatus and time-consuming procedures.

**MATERIALS AND METHODS**

Serum hepatitis-associated antigen (HB Ag). Plasma from HB Ag carriers or patients was used as source of antigen.

Assay system for HB Ag. The discontinuous counterimmunoelectrophoresis (DCIE) method used in this study has been described in detail elsewhere (12). In brief, all experiments were carried out with anode and cathode reservoirs containing 600 ml of Veronal buffer (pH 8.6, ionic strength 0.075). A constant potential of 200 v was applied for 60 min. Slides used were Kodak lantern cover glass slides (8.3 by 10.2 cm), and each was coated with 15 ml of 1% agarose (Seakem). The slides were made with agarose containing 0.015 μM of Veronal buffer, pH 8.6. HB Ag was serially diluted and tested against undiluted antiserum, which was obtained from a patient with hemophilia.

PEG 6000 and insoluble PE 60. PEG 6000, obtained from the Union Carbide Co., was used as described below. PE 60 was provided by the Monsanto Co., St. Louis, Mo., in the form of a 100-mesh powder. It is an insoluble cross-linked copolymer of isobutylene maleic anhydride. A 1% suspension in distilled water was placed on a rotary shaker for 15 min and then centrifuged at 1,500 X g for 5 min. The supernatant fluid was discarded along with a small amount of nonsedimentable or nonwettable product. The packed PE 60 was then washed twice with distilled water. After the last washing, the PE 60 was resuspended in 10-m1 samples in distilled water so that each sample contained 100 mg. The samples were centrifuged, and the supernatant fluids were discarded, leaving 100 mg of packed PE 60 ready for use.

Protein. Protein was determined by the Lowry method (9).

Cellulose acetate electrophoresis. The plasma was subjected to electrophoresis on strips which were then stained with Ponceau Red and scanned on a Beckman microzone densitometer.

**RESULTS**

Partial purification of HB Ag by PEG. The purification of plasma proteins by precipitation with PEG has been well delineated (11). Polson et al. demonstrated that the alpha globulins are precipitated by 6 to 8% PEG at pH 4.6, leaving the major proportion of albumin and of beta and gamma globulins in the supernatant fluid. HB Ag is associated with the alpha fractions of plasma.
(1, 2). On the basis of these properties, the following procedure was developed. A 100-ml amount of plasma was magnetically stirred at 25 C while 1 N HCl was slowly added, and pH 4.6 was reached and maintained. An 8-g amount of PEG powder was slowly added with continued stirring. After 10 min, a massive precipitate formed which was sedimented by centrifugation at 1,500 × g for 10 min. The supernatant fluid was collected by aspiration. To the sediment, which occupied a volume of 30 ml, 70 ml of distilled water was added, and the material dissolved after being vigorously mixed for 5 to 10 min. Plasma electrophoresis cellulose acetate strips were examined on a microzone densitometer to determine the percentages of different proteins removed by the purification method. Figure 1 shows the densitometric tracings of untreated plasma and the PEG eluate. Table 1 shows the results in terms of HB Ag titer, protein concentration, and distribution of each protein fraction.

No detectable protein or HB Ag was removed by acidification of the plasma to pH 4.6 with HCl. After addition of PEG and sedimentation of the precipitate, the supernatant fluid was free of detectable HB Ag. This latter fluid contained excess PEG which interfered with the Lowry test, and the amount of protein in this supernatant fluid was not measured. The precipitate was dissolved in water to make a final volume of 100 ml which contained 100% of the HB Ag, but only 12.5% of the original plasma proteins, 2 mg of albumin per ml, 3 mg of alpha-globulin per ml, and 5 mg of gamma globulin per ml. The results of this experiment were readily reproduced many times.

**Concentration and purification of HB Ag on PE 60.** With the removal of the major proportion of albumin and globulins from the plasma, the adsorption of HB Ag on PE 60 was found to be facilitated, as expected (13). A typical experiment is described.

A 100-ml amount of plasma was precipitated by PEG and the sediment redissolved as outlined above. To this solution, containing 100% of the HB Ag but only 12.5% of the plasma proteins, 300 mg of PE 60 was added. The pH was held at 5.0 and the temperature at 25 C. PE was sedimented at 1,500 × g for 5 min. The supernatant fluid was sampled for assay and discarded, and the sedimented PE 60 was resuspended in 10 ml of glycine buffer at pH 11.5 (0.05 M glycine, 0.15 M NaCl–NaOH). Viruses were eluted from this PE at basic pH levels in the presence of salts (13). After mixing for 5 min on a rotary shaker, the tube was centrifuged, and the supernatant fluid was collected and assayed.

To re-concentrate the eluate onto fresh PE, it had to be diluted in distilled water to render it isotonic. Under these conditions, HB Ag could be re-concentrated on fresh PE, leaving other soluble proteins in the supernatant fluids. In practice, 9 ml of the initial 10-ml eluate was diluted to a final volume of 100 ml in distilled water, and the sample was treated with PE as described above. Repeated treatments consistently yielded virtually all of the HB Ag in successive eluates with fewer and fewer plasma proteins. Table 2 shows typical

**Table 1. Purification of hepatitis B antigen (HB Ag) plasma with polyethylene glycol (PEG)**

<table>
<thead>
<tr>
<th>Samples</th>
<th>HB Ag titer</th>
<th>Protein (mg/ml)</th>
<th>Protein fractions (mg/ml)</th>
<th>Gamma globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, untreated HB Ag plasma</td>
<td>1:40</td>
<td>79.9</td>
<td>26 5.6 13 9.3 9.3 16.7</td>
<td></td>
</tr>
<tr>
<td>Supernatant fluid after acidification to pH 4.6</td>
<td>1:40</td>
<td>80.0</td>
<td>26 5.7 13 9.3 9.3 16.7</td>
<td></td>
</tr>
<tr>
<td>Supernatant fluid after PEG treatment</td>
<td>&lt; 1:1</td>
<td>ND*</td>
<td>ND ND ND ND ND ND</td>
<td></td>
</tr>
<tr>
<td>PEG eluate</td>
<td>1:40</td>
<td>10.0</td>
<td>2 0 3 0 0 5</td>
<td></td>
</tr>
</tbody>
</table>

* Excess PEG in supernatant fluid reacted with Lowry reagents. ND, Not done.
TABLE 2. Reconcentration of hepatitis B antigen (HB Ag) on polyelectrolyte (PE) 60

<table>
<thead>
<tr>
<th>Sample</th>
<th>Titer</th>
<th>Protein* (g/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma (100 ml)</td>
<td>1:40</td>
<td>8.0</td>
</tr>
<tr>
<td>Polyethylene glycol precipitate dissolved in water to make a final volume of 100 ml</td>
<td>1:40</td>
<td>100 1.0</td>
</tr>
<tr>
<td>First PE treatment 100-ml supernatant fluid</td>
<td>&lt; 1:10</td>
<td>0.93</td>
</tr>
<tr>
<td>10-ml eluate</td>
<td>1:320</td>
<td>80 0.22</td>
</tr>
<tr>
<td>Second PE treatment 100-ml supernatant fluid</td>
<td>&lt; 1:10</td>
<td>0.02</td>
</tr>
<tr>
<td>10-ml eluate</td>
<td>1:320</td>
<td>80 0.001</td>
</tr>
<tr>
<td>Third PE treatment 100-ml supernatant fluid</td>
<td>&lt; 1:10</td>
<td></td>
</tr>
<tr>
<td>10-ml eluate</td>
<td>1:320</td>
<td>80 &lt; 0.001</td>
</tr>
</tbody>
</table>

* Sensitivity of the Lowry test in our hands was 100 μg/ml.

results of three such serial purifications by PE 60. A titer of 1:320 was obtained in the PE eluates, which represents at least 80% recovery of HB Ag with an over 1,000-fold purification. Figure 2 compares the electrophoretic tracing of the first PE eluate with that of the starting plasma.

DISCUSSION

Concentration and purification procedures in current use (3–8, 10) employing cesium chloride density gradient centrifugation, electrophoresis, or column chromatography are complicated and time consuming. For example, Gerin et al. (6), employing cesium chloride density gradient centrifugation, reported that even after two-step isopycnic banding with a first centrifugation run of 11 hr and a second run of 22 hr, the HB Ag obtained was contaminated with normal, human serum proteins. Similar results were obtained by Dreesman et al. (4). In the current study we have demonstrated a simple two-step procedure for concentration of HB Ag, with consequent gross reduction of normal, human serum proteins. The HB Ag is first precipitated with PEG and subsequently adsorbed on and eluted from insoluble PE 60, all in less than 3 hr. The prior use of our procedure before the application of more sophisticated techniques would be expected to yield HB Ag preparations of greater purity with less effort.

Another application of our procedure is in the clinical laboratory. If the plasma from donor blood is first concentrated before testing, then fewer blood specimens containing HB Ag in low concentration would be missed by some of the less sensitive testing procedures in use. We are presently adapting the concentration and purification procedure to 10-ml volumes so that it can be conducted in the clinical laboratory with a minimum of apparatus and time.

ACKNOWLEDGMENTS

This investigation was supported by research contract NIH-2354 and research grant HE-05435 from the National Heart and Lung Institute and by research contract DADA 17-67C-7004 from the U.S. Army Medical Research and Development Command.

LITERATURE CITED


